IDENTIFICATION, ISOLATION AND IMPACT OF GAMMA RADIATION ON DOUBLESEX GENE OF *BACTROCERA ZONATA*. (SAUNDERS)(DIPTERA: TEPHRITIDAE).

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INTRODUCTION

Sex-determining cascades in insects constitute an intensively studied system that evolves from bottom to top (Wilkins, 1995). This cascade has been thoroughly studied in *Drosophila* and is composed of four main genes beginning with sex-lethal (Sxl) on top, transformer (Tra) and transformer-2 (Tra-2) in the middle and doublesex (dsx) on the bottom. In *Drosophila*, initiation of the sex-determining cascade is primarly determind by the ratio of x- chromosomes (X) and sets of autosomes (A) (Cline, 1993). Sxl is the gene that translates this ratio. When this ratio is 1.0, Sxl is turned on only in females Sxl, an RNA binding protein, regulates the productive splicing of mRNAs from the down stream gene Tra of the cascade (Boggs *et al.*, 1987; Inove *et al.*, 1990).

The transformer (Tra) protein acting in cooperation with the Tra-2 protein (which is expressed in both sexes) activate the female-specific splicing of the pre-mRNAs from dsx, the last gene in the cascade, leading to the production of the dsx^F protein (Hodgkin,1989).

In males, where the X/A ratio has a value of 0.5, the Sxl gene remains inactive. The lack of Sxl protein leads to the default splicing and production of non-functional truncated Tra protein. Its absence results in the default of male-specific splicing of dsx pre-mRNAs and finally the production of the dsx^M protein. The two proteins dsx^F and dsx^M are acting as transcription factors that regulate the sex specific expression of downstream genes. In a number of dipteran species, it has been shown genetically and cytogenetically that it is the presence of a dominant male determiner, often carried on the Y-chromosome, which determines the gender of the fly. This suggests that sex-determination in non-drosophilid species probably differes in the initial signal/or the master switch. Indeed, homologues of the gene Sxl were isolated in

a number of insects, *Chrysomya rufifacies* (Muller-Holtkamp, 1995), *Megaselia scalaris* (Sievert *et al.*, 1997), *Musca domestica* (Meise *et al.*, 1998), *Ceratitis capitata* (Saccone *et al.*, 1998), but appears that they donot have a sex-switch function.

In any of these species. The Sex-lethal gene generates the same product in both sexes (Ruiz *et al.*, 2003). On the contrary, dsx orthologous is characterized in a variety of distantly related species such as *Bactrocera tryoni*, (Shearman and Formmer, 1998), *M. scalaris* (Kuhn *et al.*, 2000) *C. capitata* (Saccone *et al.*, 2000). *M. domestica* (Hediger *et. al.*, 2004) and *Bombyx mori* (Suzuki *et al.*, 2001) to maintain their sex-determination function, supporting the Wilkins hypothesis that "masters change and slaves remain" (Wilkins, 1995). The fruit fly *Bactrocera zonata* (*Dacus zonata*) is a serious pest of fruits in many parts of the world. *B. zonata* larvae feed exclusively on fruits, causing severe damage to crops. *B. zonata* was recorded for the first time in Egypt, in 1999 where it caused severe damage to a wide range of fruits such as guava, peach and apricot (EL-Minshawy, 1999). Ionizing radiation technology, is used to induce dominant lethal genes causing reduction of offspring, introduced a new technique for insect control called Sterile Insect Technique (SIT) (Bushland and Hopkins 1951). The SIT method has been greatly advanced by the development of genetic sexing strains (GSS), which produce only male flies (Hendrichs *et al.*, 1995). The development of various gene transfer techniques in a range of insect pest genomes provides significant opportunities for further improvement of SIT by producing transgenic sexing strains (Atkinson *et al.*, 2001). For this reason, molecular mechanisms regulating the expression of sex-specific and sex- determining genes in insect pests have received great attention in recent years. It is imperative to determine a radiation dose developing sterility in flies but otherwise they remain healthy and vigorous in their mating behaviour. Huque and Ahmad (1966) reported that pupae of *Dacus zonatus* (*B.zonata*) when exposed to 7-9 Krad (GY), it develops sterility in adult stages without showing any deleterious effect on longevity and mating behaviour of the flies. The objectives of this work is to: investigate the impact of ionizing radiation on dsx gene , identify, isolate and characterize the sex-determing gene (dsx) in *B.zonata* and compare its homology with *Drosophila melanogaster* (dsx) and other dipteran's species.

MATERIAL AND METHODS

The infested Guava fruits were collected from Giza Governorate. The full grown larvae naturally jump to the sand where they pupate. Two days before eclosion, pupae of *B. zonata* were irradiated using gamma cell (Co_{60} source), model 220, installed at the Department of Nuclear Physics of Atomic Energy Authority, ARE. Pupae were exposed to 100, 120 & 150 GY of gamma radiation. For each dose , 100 pupae were used.

SDS PAGE Analysis

Denaturing SDS-PAGE was carried out in a vertical electrophoresis unit (large cell, Biometra) using 10% separating gel and 4% stacking gel with a continuous Tris-glycine running buffer system (50mM, pH 8.3) (Laemmli, 1970). Samples (10µl) from tissue supernatant were diluted with equal volume of 2x treatment buffer and boiled for 5 minutes. Gel was run at 60 mA constant current for 3 hours. The subunits of the refractionated samples protein were visualized by staining the gel using Comassie Brilliant Blue.

RNA Isolation and RT. PCR Analysis

Total RNA was extracted from both male and female adults using Gentra Purescript for RNA Kit.(Life Trade company)

1 µg of total RNA was reverse transcribed with AMV (Promega) according to the manufacturer's directions and about $1/20^{th}$ of the reaction was used for PCR reactions in 25 µl total volume (Pharmacia Taq) with the following forward(dsx1) & reverse (dsx2)primers.

dsx1-(CCGTCGTCTACTTCAGGAGC) dsx2-(TGACGGGACTATTCGTTTACG)

The primers were desiegned from the sequence of *B. tryoni* female-specific doublesex (dxs) at 760 bp & 1014 bp and male-specific doublesex (dsx) at 926 bp and 1324 bp. The thermal cycle program was set to 40 cycles at 94°C denaturation, 58°C annealing and 72°C elongation for 40 seconds for each step, using Perkin Elmer Gene Amp 9600. PCR products were recovered, and sequenced by automated DNA sequencing reactions, which were performed using sequencing ready reaction kit (Applied Biosystems, USA) in conjunction with ABI-PRISM and ABI-PRISM big dye terminator cycler.

RESULTS AND DISCUSSION

As ionizing radiation penetrates a living cell, it collides randomly with atoms and molecules in its path, giving rise to ions, free radicals, and other molecular alterations that may injure the cell. Any molecule in the cell can be altered by radiation, but DNA is the most critical biological target because of the limited redundancy of the genetic information it contains. A dose of radiation that is large enough to kill the average dividing cell causes hundreds of lesions in the cell's DNA molecules. Most of such lesions are repairable, but those produced by a densely ionizing radiation (such as a proton or an alpha particle) are generally more complex and less repairable than those produced by a sparsely ionizing radiation (such as an

X-ray or a gamma ray). Any damage to DNA that remains unrepaired or is improperly repaired may result in a mutation or chromosome aberration, and both of these types of effects appear to rise in frequency in proportion to any increase in the radiation dose.

Polymorphism of total soluble protein patterns

Total soluble proteins of *Drosophila melanogaster*, non-irradiated and irradiated adult female of *B. zonata* are size fractionated into 38 bands on 10% SDS PAGE gel Fig.(1).

Relative mobility (Rm) of these indicated bands were measured compared to the molecular weight (MW) marker lane. There are 8 common bands in both *D. melanogaster* & *B. zonata* with MW. 133, 87, 78, 76, 72, 45, 43 & 14 KDa, respectively whil the bands MW 74, 41 &20 KDa, respectively are specific bands for *Drosophila melanogaster* only. Also, there are 10 common bands appeared only in both irradiated & non-irradiated *B. zonata* with MW 106, 98, 94.9, 90.4, 83, 81, 69, 63, 40 & 37 KDa. (Table 1).

The present investigation revealed that, the exposure of the fruit fly *B. zonata* to gamma rays caused the disappearance of some bands and the apearance of specific protein bands. The bands with MW 180.87, 167.08, 34, and 23 KDa disappeared only in fractionated protein samples isolated from *B. zonata* irradiated with 100, 120, &150 GY. These results may be due to gene supression as a response to DNA damage & unable to repair the damage.

On the otherhand, many bands appeared in fractionated protein samples isolated from the irradiated *B. zonata* with different doses. The bands with MW 143.9, and 129 KDa appeared with *B. zonata* irradiated with 100, 120 & 150 GY. Also, the bands with MW 202.31, 150.12, 126.14, 122.13, 115.34, 104, 33, 29, 22, 20, &17 KDa. appeared only in fractionation of protein samples isolated from *B. zonata* irradiated with 150 GY. The results revealed that two specific proteins appeared in *B. zonata* irradiated with 100 & 120GY of gamma radiation. Also, eleven specific proteins appeared only in *B. zonata* irradiated with 150 GY (Fig.1). The appearance of these bands may be due to the increase of DNA repair enzyme expession.

RNA isolation and RT-PCR analysis

Two sets of RT. PCR reactions were performed in males and females with primers dsx1 and dsx2 which were designed to amplifly 300bp fragment in females only and 400bp fragment in males only (Fig.2).

TABLE (I)

Relative mobility and Molecular weight of SDS protein bands detected in the whole homogenates of *Bactrocera zonata* (Saunders) adults female emerged from pupae irradiated with different doses of gamma radiation in comparison with non-irradiated adults of *B. zonata* & *D. melanogaster*.

Band No	Rm	Molecular weight	Drosophila	Non-irradiated Bactrocera	Irradiated Bactrocera		
					100 GY	12 0 GY	15 0 GY
1	0.045	202.31	-	-	-	-	+
2	0.077	180.87	-	+	-	+	+
3	0.103	167.08	-	+	-	-	+
4	0.139	150.12	-	-	-	-	+
5	0.154	143.91	-	-	+	+	+
6	0.178	133.90	+	+	+	+	+
7	0.18	129	-	-	+	+	-
8	0.198	126.14	-	-	-	-	+
9	0.209	122.13	-	-	-	-	+
10	0.228	115.34	-	-	-	-	+
11	0.244	106.48	-	+	+	+	+
12	0.25	104	-	-	-	-	+
13	0.26	98	-	+	+	+	+
14	0.285	94.9	-	+	+	+	+
15	0.287	90.4	-	+	+	+	+
16	0.288	87	+	+	+	+	+
17	0.289	83	-	+	+	+	+
18	0.297	81	-	+	+	+	+
19	0.308	78	+	+	+	+	+
20	0.31	76	+	+	+	+	+
21	0.367	74	+	-	-	-	-
22	0.378	72	+	+	+	+	+
23	0.407	69	-	+	+	+	+
24	0.42	63	-	+	+	+	+
25	0.47	45	+	+	+	+	+
26	0.48	43	+	+	+	+	+
27	0.5	41	+	-	-	-	-

Table (I) continued

Band No	Rm	Molecular weight	Drosophila	Non-irradiated Bactrocera	Irradiated Bactrocera		
					100 GY	100 GY	100 GY
28	0.561	40	-	+	+	+	+
29	0.579	37	-	+	+	+	+
30	0.61	34	-	+	+	+	-
31	0.63	33	-	-	-	-	+
32	0.66	29	-	-	-	-	+
33	0.736	23	-	+	+	-	+
34	0.75	22	-	-	-	-	+
35	0.79	20	+	-	-	-	+
36	0.81	18	-	+	+	+	-
37	0.83	17	-	-	-	-	+
38	0.95	14	+	+	+	+	+

The results indicated that Bzdsx is transcribed into two different transcripts in adult male and female individuals, respectively. Bzdsx pre-mRNA could be spliced alternatively in a sex-specific manner. Kuhn *et. al.*, 2000 reported that the production of sex-specific transcripts in *Drosophila* dsx gene is differentially spliced. In males, exon 4 is skipped by default, and instead the downstream exons 5 and 6 are included in the mature transcript. In females, the presence of the splicing regulatory activities of Tra/Tra-2 genes promote the incorporation of exon 4 in the mature transcript. This pattern of sex specific splicing is also observed in the Queens land fruit fly *Bactrocera tryoni*, *Bactrocera oleae* and the phorid fly *Megaselia scaleris* where it occurs in equivalent positions of the corresponding dsx genes (Shearman and Frommer, 1998). Moreover, Pane *et al.* (2002) demonstrated that female-specific splicing of the dsx gene in the Mediterranean fruit fly *Ceratitis capitata* depends on the activity of the tra gene homologie. The presence of putative Tra/Tra-2 binding sites in the female-specific exon of dsx gene in *Bactrocera* and *Megaselia* gives further support to the notion that female exon selection by activation is common in dipteran insects. Fragment of the female dsx gene was recovered with RT-PCR and sequenced. Fragment of the irradiated female dsx gene with 150GY was recovered with RT. PCR and sequenced. The sequences revealed that irradiation treatment causes a mutation which is known as a substitution mutation because one Guanine nucleotide is replaced by a cytosine nucleotide (Fig.3). The

sequences of the fragment showed a 99%, 98%, 97% and 76% homology at the nuclotide level to the corresponding fragment of Bddsx, Bodsx, Btdsx and Dmdsx female, respectively (Fig.4a) and 92%, 91% ,90% and 76% similarity at the nucleotide level to the corresponding fragment of Bddsx, Bodsx, Btdsx and Dmdsx male, respectively. (Fig.4b).

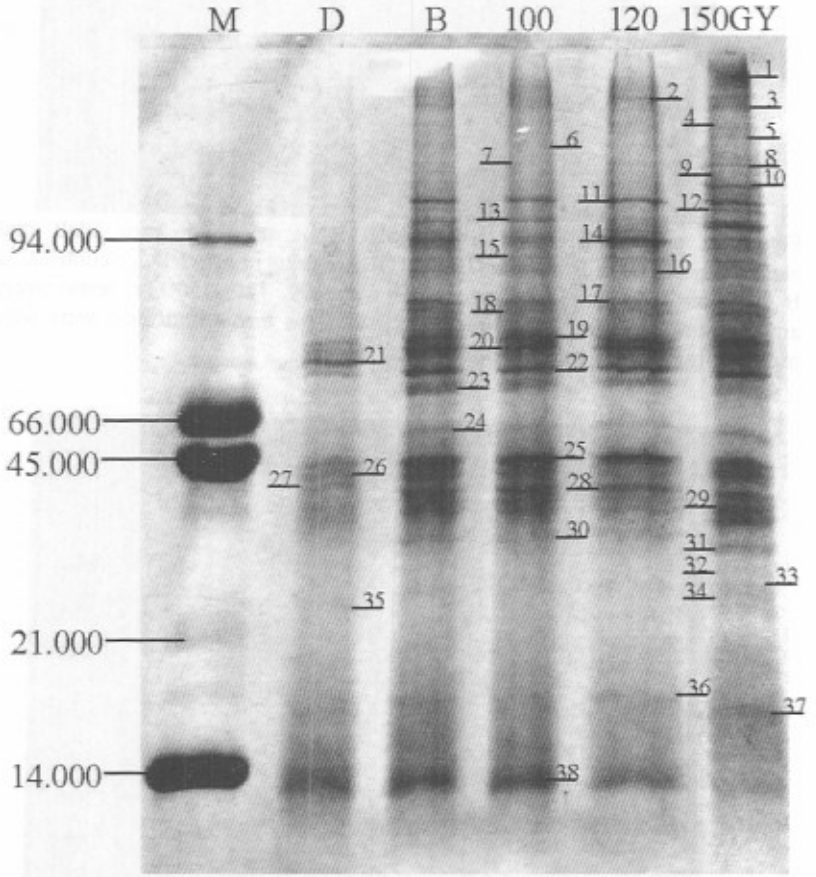

Figure 1: SDS-Polyacrylamide gel of denatured protein patterns, in the irradiated and non-irradiated female adults of *B. zonata*(B) and *D. melanogaster*(D). Lane 1 represents *D. melanogaster,* Lane 2 represents *B. zonata* (non-irradiated) Lane 3-5 represent irradiated *B. zonata* with 100, 120. 150Gy, respectively. M represent the molecular weights of marker bands indicating on the left side of the gel. Protein band numbers were indicating on the gel

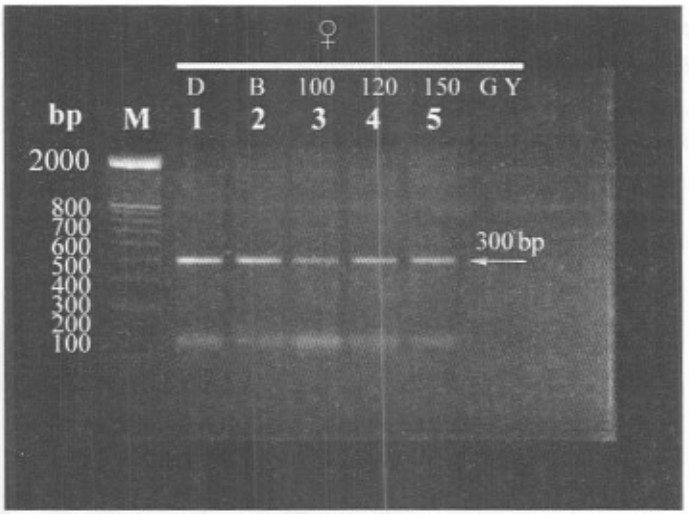

Figure 2: a- RT-PCR analysis of total RNA prepared from adult male *D. melanogaster* (D) & *B. zonata* (B). M (DNA marker) Lane 1 D ♀ (control), Lane 2 B ♀ (control) Lanes 3-5 (irradiated B with 100, 120, 150 Gy, respectively) for amplification in lanes 1-5, primers dsx_1, dsx_2 (as arrow indicates) were used and produce fragment of size 300 bp.

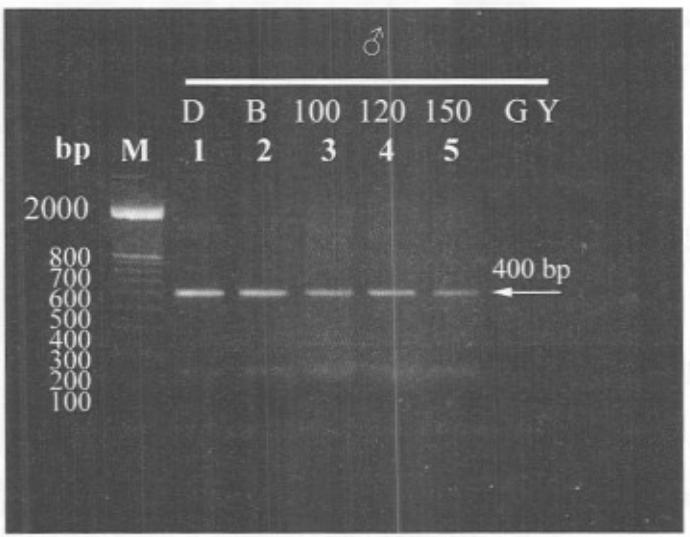

Figure 2: b- RT-PCR analysis of total RNA prepared from adult male *D. melanogaster* (D) & *B. zonata* (B). M (DNA marker) Lane 1 D ♂ (control), Lane 2 B ♂ (control) Lanes 3-5 (irradiated B with 100, 120, 150 Gy, respectively) for amplification in lanes 1-5, primers dsx_1, dsx_2 (as arrow indicates) were used and produce fragment of size 400 bp

S3

ATTCCTTTAGCTCAGATGTCTTTTTAGAGCATTGTCAAAAACTATTGG
AGAAATTTCGATATCCTTGGGAGATGATGCCATTAATGTATGTGATA
TTAAAAGATGCTGGGGCAGATATTGAAGAGGCTTCAAGACGCATTGA
GGAAGGCCAACATGTCGTAAACGAATACTCCCGT

S3: 1

ATTCCTTTAGCTCAGATGTCTTTTTAGAGCATTGTCAAAAACTATTGG
AGAAATTTCGATATCCTTGGGAGATGATGCCATTAATGTATGTGATA
TTAAAAGATGCTGGGGCAGATATTGAAGAGGCTTCAAGACGCATTGA
GGAAGGCCAACATGTCGTAAACCAATACTCCCGT

Figure 3: S3 represents nucleotide sequence of the non irradiated *B. zonata* female double sex (Bzdsx) fragment. S3: 1 show the mutation in irradiated *B. zonata* female double sex (Bzdsx).

The results show that, dsx gene in *B. zonata* appears to be considerably conservative in structure and in all non-drosophilid species studied. These results agreed with Kuhn *et al.* (2000) who reported that the primary SxL gene and secondary sex-determing Tra gene are not conserved. Subsequently, dsx gene is conserved among flies even when it belongs to such distantly related groups as Schizophora (*Drosophila, Bactrocera*) and Aschiza (*Megaselia*). The conservation of functional domains in dsx of the silk moth, *Bombyx mori*, indicated that this step in the sex-determing pathway is conserved in an even wider range of different insect orders (Suzuki *et al* ., 2001). Also, "studies of the sex-determing cascades in a variety of organisms from insects to mammals provide great support to the hypothesis proposed by Wilkins (1995) that genetic hierarchies evolve from "bottom up". According to this hypothesis the final gene of a regulatory cascade is the most ancient one, remaining quite conserved but possibly controlled by different upstream gene regulators in different organisms. In *Drosophila* ,the Sxl gene is at the top of the genetic cascade controlling sex determination and although it remains conserved in sequences among a variety of dipterans it has not acquired a sex-determining function apart from that in the drosophilids. In contrast, the dsx gene, which is placed at the bottom of that genetic cascade, is highly conserved in the insects in which it has been characterized so far, not only with respect to sequence but also in relation to its sex-specific regulation.Therefore, the dsx gene but not the Sxl gene can be used for the development of molecular tools that will improve the SIT technique used for pest control" (Lagos *et al* ., 2005).

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Bzdsx                                                                AT-T      3
Btdsx       TATTGCCCATATCGGTGGTAGGACGCAAACCATCTCTGCATCCCAATGGAGTAAATAT-T    840
Bddsx       TATTGCCCATATCGGTGGTAGGTCGCAAACCATCTCTGCATCCCAATGGAGTAAATAT-T    885
Bodsx       TATTGCCCATATCGGTGGTAGGTCGCAAACCATCTCTGCATCCTAATGGAGTAAATAT-T   2058
Dmdsx        --TT-CGCGTAAAAGTGTTAG-TCAAAAACCATTTTTTTAAACTTTTTTTTTATGTGTGT   2187
                 :    .    :    .    :    .    :    .    :    .    :  2520
Bzdsx       CCTTT----AGCTCA-GATGTCTTTTT---AGA----GCATTGTCA-AAAAC-TATTGGA     49
Btdsx       CCTTT----AGCTCAAGATGTCTTTTT---GGA----GCATTGTCA-AAAAC-TATTGGA    887
Bddsx       CCTCT----AGCTCAAGATGTCTTTTT---AGA----GCATTGTCA-AAAAC-TATTGGA    932
Bodsx       CCTTT----AGCTCAAGATGTCTTTTT---AGA----ACATTGTCA-AAAAC-TATTGGA   2105
Dmdsx       GTTTTCCAAAGCGGAAAATTTTTTTTTCCCGGATACAGCACAATAACAAAACGTAAAGCT   2247
              :    .    :    .    :    .    :    .    :    .    :  2580
Bzdsx       GAAATTTCGAT-ATCCT--TGGGAGAT---GA--TGC-CATTAATG--TA-TGTGATATT     97
Btdsx       GAAATTTCGAT-ATCCA--TGGGAGAT---GA--TGC-CATTAATG--TA-TGTGATATT    935
Bddsx       GAAATTTCGAT-ATCCA--TGGGAGAT---GA--TGC-CATTAATG--TA-TGTGATATT    980
Bodsx       GAAATTTCGAT-ATCCA--TGGGAGAT---GA--TGC-CATTAATG--TA-TGTGATATT   2153
Dmdsx       GCAATTTTTTTTATACAAATAAAAAATACCGAAATGCACAT-AATGAGTAATAT-ATATT   2305

              :    .    :    .    :    .    :    .    :    .    :  2640
Bzdsx       AAAAGATGCTGGGGCAGATATTGAAGAGGCTTCAAG-AC-GCATTGAGG---AAGGCCAA    152
Btdsx       AAAAGATGCTGGGGCAGATATTGAGGAAGCTTCAAG-AC-GCATTGAGG---AAGGCCAA    990
Bddsx       AAAAGATGCTGGGGCAGATATTGAAGAGGCTTCAAG-AC-GCATTGAGG---AAGGCCAA   1035
Bodsx       AAAAGATGCTGGGGCAGATATTGAAGAGGCTTCAAG-AC-GCATTGAGG---AAGGCCAA   2208
Dmdsx       AACA-A--CAAAG--AGATA---AACCAAC--CAAGGACTGAATCGATACCTAACTAAAA   2355
              :    .    :    .    :    .    :    .    :    .    :  2700
Bzdsx       CATGTCGTAAACGAATACTCCCGT                                        176
Btdsx       CATGTCGTAAACGAATACTCCCGTCAACACAATCTGA-ATATATA-----TGA-CGGGGG   1043
Bddsx       CATGTCGTAAACGAATACTCCCGTCAACACAATCTGA-ATATATA-----TGA-CGGGGG   1088
Bodsx       CATGTCGTAAACGAATACTCCCGTCAACACAATCTGA-ATATATA-----TGA-CGGGGG   2261
Dmdsx       -AAGCAAGAAATGCATAA-CCCACTAAAA-AAGATGATATATATCCTTCTTGAACGAAGC   2412
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Figure 4:a- Multiple alignment of dxs gene sequences of *B.zonata* (Bzdxs), *B. dorsalis* (Bddxs), *B. oleoe* (Bodsx), *B. tryoni* (Btdsx) and *D. melanogaster* (Dmdsx) female.

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                    .    :    .    :    .    :    .    :    .    :    .    :   2520
Bzdsx          ATTCCTTTAGCTCA-GATGTCTTTTT---------AGAGCATTGTCAAAAACTATTGG     48
Btdsx        ATATTCCTTTAGCTCAAGATGTCTTTTT---------GGAGCATTGTCAAAAACTATTGG    886
Bddsx        ATATTCCTCTAGCTCAAGATGTCTTTTT---------AGAGCATTGTCAAAAACTATTGG    931
Bodsx        ATATTCCTTTAGCTCAAGATGTCTTTTT---------AGAACATTGTCAAAAACTATTGG   2104
Dmdsx        GTTTTCCAA-AGCGGAAAATTTTTTTTTCCCGGATACAGCACAATAACAAAACGTAAAGC   2246

                    .    :    .    :    .    :    .    :    .    :    .    :   2580
Bzdsx        AGAAATTTCGAT-ATCCT--TGGGAGAT---GA--TGC-CATTAATG--TA-TGTGATAT     96
Btdsx        AGAAATTTCGAT-ATCCA--TGGGAGAT---GA--TGC-CATTAATG--TA-TGTGATAT    934
Bddsx        AGAAATTTCGAT-ATCCA--TGGGAGAT---GA--TGC-CATTAATG--TA-TGTGATAT    979
Bodsx        AGAAATTTCGAT-ATCCA--TGGGAGAT---GA--TGC-CATTAATG--TA-TGTGATAT   2152
Dmdsx        TGCAATTTTTTTTATACAAATAAAAAATACCGAAATGCACAT-AATGAGTAATAT-ATAT   2304

                    .    :    .    :    .    :    .    :    .    :    .    :   2640
Bzdsx        TAA-----AAGATGCTGGGGCAGATATTGAAGAG-GCTTCAAGACGCATTGAGGAAGGCC    150
Btdsx        TAA-----AAGATGCTGGGGCAGATATTGAGGAA-GCTTCAAGACGCATTGAGGAAGGCC    988
Bddsx        TAA-----AAGATGCTGGGGCAGATATTGAAGAG-GCTTCAAGACGCATTGAGGAAGGCC   1033
Bodsx        TAA-----AAGATGCTGGGGCAGATATTGAAGAG-GCTTCAAGACGCATTGAGGAAGGCC   2206
Dmdsx        TAACAACAAAGA-GATAAACCAACCA---AGGACTGAATCGATACCTAACTAAAAAAGC-   2359

                    .    :    .    :    .    :    .    :    .    :    .    :   2700
Bzdsx        AACATGTCGTAAACGAATACTCCCGT                                      176
Btdsx        AACATGTCGTAAACGAATACTCCCGTCAACACAATCTGA-ATATAT-----ATGA-CGGG   1041
Bddsx        AACATGTCGTAAACGAATACTCCCGTCAACACAATCTGA-ATATAT-----ATGA-CGGG   1086
Bodsx        AACATGTCGTAAACGAATACTCCCGTCAACACAATCTGA-ATATAT-----ATGA-CGGG   2259
Dmdsx        AA---G----AAATGCATAACCCACTAAA-A-AAGATGATATATATCCTTCTTGAACGAA   2410
                    .    :    .    :    .    :    .    :    .    :    .    :   276
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Figure 4:b- Multiple alignment of dxs gene sequences of *B.zonata* (Bzdsx), *B. tryoni* (Btdsx), *B. dorsalis* (Bddsx), *B. oleoe* (Bodsx), and *D. melanogaster* (Dmdsx) male

Moreover, the identification of female and male specific transcripts of *B. zonata* dsx represents an important step toward the understanding of the sex differentiation process in *B. zonata* and will facilitate the development of genetic tools to induce male sterility or manipulate sex ratios in fruit flies, for instance by constitutively expressing the female specific form of dsx gene in the male gonads or by inducing the sex specific splicing as a dominant lethal trait.

Construction of cDNA library, isolating and sequencing full length gene is necessary for molecular characterization for the hypersensitive, conservative and functional regions in the Bzdsx gene which allow more understanding of the mode of action and control of dsx gene expression.

SUMMARY

In Egypt, *Bactrocera zonata* caused considerable damage and inflicted significant economic losses in fruit orchards, in the last decade, so that it is established as an economic pest. Investigation of the effect of gamma radiation on total soluble proteins using SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) revealed several protein bands from homogenate samples of irradiated and control adults of *B. zonata.* However, it was not possible to detect protein bands that show any shifts or separations between the irradiated and control adult flies. Irradiation treatment resulted in disappearance of different low molecular weight protein bands, causing ploymorphism and altering the electrophoretic patterns and densities of proteins. The alterations in total proteins of adult *B. zonata* are related to the dose of gamma radiation.

Investigation of the effect of Gamma radiation on the expression of *B. zonata* dsx gene had been performed. Two days before eclosion, mature pupae were irradiated at 100, 120, 150 GY gamma radiation, respectively as the effective recorded dose. This study include the identification, isolation and molecular characterization of the fruit fly *B. zonata* doublesex (Bz dsx) gene homologs to the *D. melanogaster* dsx gene. Total RNA has been isolated from non-radiated and radiated *B. zonata* adult flies. Fragments of the dsx were recovered with RT-PCR. The amplified products were found to be of different length in males than in females. The sequenced fragment of irradiated female with 150 GY dose revealed some sequence mutation. The gene shares a high degree of similarity in sequence and expression compared to *D. melanogaster* orthologous and appear to play a regulatory role in the sex determining cascade.

REFERENCES

ATKINSON, P. W.; PINKERTON, A. C. and O'BROCHTA, D. A. (2001): Genetic transformation systems in insects. *(Ann. Rev. Entomol. 46, 317-346).*

BOGGS,R.T.GREGOR,P.;IDRISS,S.;BELOTE,J.M.andMCKEOWN,M. (1987): Regulation of sexual differentiation in *D. melanogaster* via alternative splicing of RNA from the transformer gene. *(Cell 50,739-747).*

BUSHLAND, R. C. and HOPKINS, D. E. (1951): Experiments with screw- .worm flies sterilized with x-rays. *(J. Econ. Entomol. 44: 725-31).*

CLINE,T.W.(1993): The *Drosophila* sex determination signal: how do flies count to two? (*Trends Genet.* 9, 385-390).

EL-MINSHAWY , A. M.; EL–ERYAN , M. A. and AWAD, A.I. (1999): Biological and morphological studies on the guava fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) Found recently in Egypt. *(8th Nat. Conf. Of Pests & Dis of Veg. & Fruits in Ismailia , Egypt, 71-82).*

HEDIGER, M.; G. BURGHARDT; C. SIEGENTHALER; N. BUSER; D. HILFIKER-KLEINER; A. DÜBENDORFER and D. BOPP (2004): Sex determination in *Drosophila melanogaster* and *Musca domestica* converges at the level of the terminal regulator doublesex. *(Dev. Genes Evol. 214, 29-42).*

HENDRICH, J.; G. FRANZ and P. RENDOM (1995): Increased effectiveness and applicability of the sterile insect technique through male-only release for control of Mediterranean fruit-flies during fruiting season. *(J. Appl. Entomol. 119, 37-371).*

HODGKIN, J. (1989): *Drosophila* sex determination :a cascade of regulated splicing. *(Cell 56,905-906).*

HUQUE, H. and H. AHMAD (1966): Effect of gamma radiation on *Dacus zonatus* and *Dacus cucurbitae. (Fd Irrad. 6, A28-A32).*

INOVE, K.; K. HOSHIJIAMA; H. SAKAMOTO and Y. SHIMURA (1990): Binding of the Drosophila Sex-lethal gene product to the alternative splice site of transformer primary transcript. *(Nature 344,461-463).*

KUHN, S.; V. SIEVERT and W. TRAUT (2000): The sex-determining gene doblesex in the fly *Megaselia scalaris*: conserved structure and sex-specific splicing. *(Genome 43, 1011-2020).*

LAGOS, D.; M. F. RUIZ; L. SANCHEZ and K. KOMITOPOULOU (2005): Isolation and characterization of the *Bactrocera oleae* genes orthologous to the sex determining Sex-lethal and doublesex genes of *Drosophila melanogaster*. *(Gene. Mar. 28(348): 111-121).*

LAEMMLI, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *(Nature 227,680-685).*

MEISE, M.; D. HILFIKER-KLEINER; A. DUBENDORFER; C. BMNER; R. NOTHIGER and D. BOPP (1998): Sex-lethal, the master sex-determing gene in *Drosophila,* is not sex-specifically regulated in *Musca domestica. (Develop. Apr. 125(8): 1487-1494).*

MULLER-HOLTKAMP F. (1995): The Sex-lethal gene homologue in *Chrysomya rufifacies* is highly conserved in sequence and exon-intron organization. *(J. Mol. Evol.41, 467-477).*

PANE, A.; M. SAVEMINI; P. DELLIBOVI; C. POLITO and G. SACCONE (2002): The transformer gene in *Ceratitis capitata* provides a genetic basis for selecting and remembering the sexual fate . *(Development.129:3715-3725).*

RUIZ, M. F.; C. GODAY; P. GONZALEZ and L. SANCHEZ (2003): Molecular analysis and developmental expression of the Sex–lethal gene of *Sciara ocellaris* (Diptera: Nematocera). *(Gene Expr. Patterns 3, 341 – 346).*

SACCONE,G.; L. PELUSIO; D. ARTIACO; E. GIORDANO; D. BOPP and L. C. POLIO (1998): The *Ceratitis capitata* homolougue of the *Drosophila* sex-determing gene Sex-lethal is structurally conserved,but not sex- specifically regulated. *(Development 125, 1495-1500).*

SACCONE,G.; L. PELUSIO; D. ARTIACO; E. GIORDANO; D. BOPP and L. C. POLIO (2000): Sex determination in Medfly: a molecular approach. *(In: Tan, K. H. (Eds.) Area-wide control of Fruit flies and other insect pests. Penerbit Universiti Sains Malaysia, Penang pp.491-496).*

SHEARMAN, D. C. A. and M. FORMMER (1998): The *Bactrocera tryoni* homologue of the *Drosophila melanogaster* sex-determination gene doublesex. *(Insect Mol. Biol. 7, 1-12).*

SIEVERT, V.; S. KUHN and W. TRANT (1997): Expression of the sex-determing cascade genes Sex-lethal and doublesex in the phorid fly *Megaslia scalaris. (Genome 40, 211-214).*

SUZUKI, M. G.; F. OHBAYASHI; K. MITA and T. SHIMODA (2001): The mechanism of sex-specific splicing at the *doublesex* gene is different between *Drosophila melanogaster* and *Bombyx mori. (Insect Biochem. Mol. Biol. 31, 1201-1211).*

WILKINS, A.S. (1995): Moving up the hierarchy :a hypothesis on the evolution of a genetic sex determination pathway. *(Bioessays 17, 71-77).*